and

(b) freezing the cell suspension at a cooling rate of about 1° to about 10° C/minute to yield a frozen cell suspension.

Remarks

Reconsideration and withdrawal of the rejections in the final Office Action mailed on May 30, 2002 and the Advisory Action dated November 18, 2002 for the above-identified application, in view of the amendments and remarks herein, is respectfully requested. Claims 53-58 are added, and claims 1 and 26 are amended. The amendments are intended to further prosecution and are not intended to concede to the correctness of the Examiner's position or to prejudice the prosecution of the claims prior to amendment, which claims are present in a continuation of the present application. Claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 are pending.

The amendment to claims 1 and 26 is supported at page 3, lines 20-22 and page 4, lines 16-19 of the specification.

Support for new claims 53 and 55 is found in originally-filed claims 1 and 31, respectively, and at page 3, lines 20-22 and page 4, lines 16-19 of the specification.

Support for new claims 54 and 56 is found in originally filed claims 1 and 31, respectively, and at page 3, line 26 of the specification.

Support for new claims 57-58 is found in originally-filed claim 26, and at page 3, line 26, page 28, lines 10-11, and Tables 2-3 in the specification.

The Examiner rejected claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-52 under 35 U.S.C. § 102(a) as anticipated by, or in the alternative under 35 U.S.C. § 103(a) as obvious over, WO 97/35472. These rejections are respectfully traversed.

WO 97/35472 discloses that the concentration of arabinogalactan (AG) in cryopreservation media is typically from between 5 and 70% w/v, preferably between 14 and 20% w/v, although it may also be between 20 and 50% w/v, e.g., preferably between 44 and 50% w/v (page 7). Optional cryopreservation agents in the media are disclosed as including DMSO, serum, and glycerol (page 7). Although WO 97/35472 indicates that the described media may be

employed with a variety of cell types including human cells (page 5) and "blood" or "immune" cells (page 10 and page 11, respectively), the only data provided in the WO 97/35478 specification is for seven lines of immortalized mammalian cells (page 13). These include three lines derived from rodent epithelial cells, a line derived from mink fibroblasts, a line derived from human fibroblasts, a line derived from bovine endothelial cells (CPAE cells), and a line derived from murine pre-neoplastic mammary cells. No blood-derived hematopoietic cells are represented in the seven lines of cells disclosed in WO 97/35472.

These seven lines were frozen in 6 different media (Table 1). For media containing AG, it is disclosed that AG was prepared as a 50% w/v concentrated stock dissolved in a buffered isotonic salt solution. This stock was used directly (medium 3, i.e., 50% AG) or in combination with other components. Medium 4 has 20% AG and 10% DMSO; medium 6 has 15% AG and 20% serum, medium 2 has 10% AG and 20% DMSO; and medium 5 has 10% AG, 10% DMSO and 20% serum. Medium 1 has 10% DMSO and 20% serum (no AG). Note that media which include DMSO or serum are not generally suitable for administration to a human due to DMSO-related toxicity or the potential for a transmissible infectious agent in serum.

With respect to immediate post-thaw viability for all cell types tested, it is disclosed that there was no difference in post-thaw viability for 4 of the media relative to "the industry standard" (cell culture medium + serum + DMSO) (page 14), however, cells frozen in media with AG and serum had reduced viability. It is also noted that there was "substantially no difference" in plating efficiency at day 1 for 6/7 of the cell types (page 14). At six days post-thaw, it is disclosed that there was "substantially no difference" between treatment groups (page 15). Table 2 shows the ranking of the media with respect to growth rates (Day 6/Day 1) for CPAE cells (media 3 > media 5 > media 2 > media 1 > media 4 > media 6). WO 97/35472 concludes that AG "can be used to replace serum in a standard freezing medium, in a formulation with DMSO, for all cell types studied" (page 15, emphases added) and that freezing in 50% w/v AG was better or equivalent to the standard media for 5/7 cell types tested (page 15). Thus, although a medium with a very high percent of AG (50%) alone was at least equivalent to standard freezing media for 5/7 cell types tested, a lower percent of AG was not able to successfully replace DMSO in a standard freezing medium.

WO 97/35472 generally discloses that the cells may be cooled or frozen during storage to about or below 4°C, for example to about -200°C. An exemplary freezing procedure is described as resuspending cells in an AG-containing freezing medium (1×10^6-1×10^7 cells/vial), aliquoted into 1.8 ml cryovials, equilibrated for about 30 minutes at 4°C, step-cooled for 18 hours at -80°C and immediately transferred to liquid nitrogen (-196°C) (page 8 and Example 2).

Clearly, WO 97/35472 fails to describe a composition comprising 1% w/v to 40% w/v AG and freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are modified *in vitro*, which does not contain DMSO or serum, or a cryopreservation method which employs such a composition, e.g., one which results in a high post-thaw survival rate for those cells, or a method which employs such a composition (claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-52). Nor does WO 97/35472 describe a cryopreservation medium comprising 1% w/v to 40% w/v of AG, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo* (claims 53 and 55). Moreover, WO 97/35472 fails to describe a cryopreservation medium comprising 1% w/v to 40% w/v of AG, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo* or a method of using such a medium (claims 54 and 56-57).

Further, WO 97/35472 fails to describe a method for preserving cells in which freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo* are cooled at a cooling rate of about 1° to about 10° C/minute (claim 58).

The Examiner is also requested to reconsider that methods and compositions useful to cryopreserve one cell type are not necessarily useful to preserve cell types, as each cell type has different biological and physical properties. For example, in the Rule 132 Declaration filed with the Amendment on August 27, 2001, executed by Dr. Allison Hubel, Dr. Hubel states that a

variety of interrelated factors influence the ability of cells to survive the stresses of freezing and thawing including (1) the composition of the cryopreservation solution; (2) the temperature history of the sample during cooling (e.g., cooling rate); and (3) the biological and biophysical characteristics of the cell/tissue being frozen (paragraph 5 of the Declaration). Dr. Hubel also states that during rapid cooling, there is insufficient time for water to leave the cell in response to the increase in extracellular solution concentration resulting from the removal of water experienced during freezing (paragraph 7 of the Declaration). Undercooling of the cell relative to the extracellular solution results in intracellular ice formation, a lethal event, and slow cooling can result in excessive dehydration of the cell that is also damaging to the cell (paragraph 7 of the Declaration). Dr. Hubel also states that the relative water content of a cell during freezing is a function of the cell type (with each cell type exhibiting its own unique biophysical characteristics) and the function of the solution composition in which the cell is suspended (paragraph 7 of the Declaration). Evidence that survival and cooling rate vary with the composition, and that different cell types have different cooling rates when present in the same freezing medium, is provided in paragraphs 8 and 9 of the Declaration.

In this regard, the Examiner is also requested to reconsider page 97 of Sputtek et al. (In: Clinical Applications in Cryobiology, CRC Press, 1991), where it is noted that the conditions employed to freeze red blood cells do not result in viable white blood cells (a copy was provided with the Amendment filed on August 27, 2001). Further, in Hubel (Transfusion Med. Rev., 11, 224 (1997)) (a copy was provided with the Amendment filed on August 27, 2001), it is disclosed that the membrane permeability parameters for a number of blood cell types including lymphocytes was found to be distinctive (see Table 1). In addition, Figure 3 in Hubel provides data showing that freshly isolated $CD34^+$ cells and cultured, transduced $CD34^+$ cells have different physical characteristics at different temperatures, including water permeability, cell volume and the osmotically inactive cell volume fraction (page 228).

Yet further evidence that different cell types have different properties in any particular cryopreservation medium is shown in Table 3 and 4 of Applicant's specification. Tables 3 and 4 show the differences in cell recovery for activated peripheral blood lymphocytes versus cultured peripheral blood lymphocytes and genetically altered peripheral blood lymphocytes versus

normal peripheral blood lymphocytes in the same AG-containing cryopreservation medium and relative to DMSO-containing medium.

In addition, the Examiner is respectfully requested to reconsider the Rule 132 Declaration enclosed with the Response filed on October 30, 2002, executed by Dr. John C. Bischof. In the Declaration, Dr. Bischof states that, prior to the filing of the above-identified application, there was still a considerable need for the development and refinement of cyropreservation solutions and protocols (paragraph 3 of the Declaration). He explains that, in particular, solutions and protocols developed for cells which were amenable to cryopreservation and employable in cellular-based therapies, frequently resulted in suboptimal levels of post thaw viability and, more often than not, employed protective agents that were harmful upon infusion (paragraph 3 of the Declaration).

Moreover, Dr. Bischof states that the fundamental physical phenomena present during freezing are strongly influenced by the composition of the freezing solution and the cell type being preserved and that protocols and solutions developed for one cell type may not be appropriate for another (paragraph 4 of the Declaration). Based on the data in WO 97/35472, Dr. Bischof concludes that one skilled in the relevant field would not have a reasonable expectation that the protocols and solutions disclosed in WO 97/35472 would be useful for other cell types and, in particular, for freshly isolated lymphocytes, hematopoietic stem cells, or *ex vivo* modified lymphocytes (paragraphs 4 and 6 of the Declaration).

Further, Dr. Bischof states that most clinical and commercial applications of cryopreserved cells or tissues require a threshold level of post thaw viability, e.g., 50% post thaw viability. Dr. Bischof concludes that WO 97/35472 provides no reasonable expectation that the use of any particular arabinogalactan containing solution would result in a threshold level of post thaw viability for cells employed in cellular-based therapies (paragraph 6 of the Declaration).

Thus, because the concentration of AG useful in a cryopreservation medium is based on the biophysical properties of each cell type, and so varies with cell type, WO 97/35272 fails to disclose or suggest a cryopreservation composition or method that would be effective to preserve freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are modified *ex vivo*, or a method of cryopreserving those cells. In particular, WO 97/35272 fails to disclose or

suggest a cryopreservation composition or method which yields a high survival rate for freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are modified *ex vivo*.

Therefore, the Examiner is respectfully requested to withdraw the § 102(a) and § 103(a) rejections of the claims.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date November 27, 2002 By _____
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Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Box RCE, Commissioner of Patents, Washington, D.C. 20231, on this 27th day of November, 2002.

Anne M. Richards
Name

Signature